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Analysis of LuPME3, a pectin methylesterase from *Linum usitatissimum*, revealed a variability in PME proteolytic maturation

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Pectin methylesterase (PME) catalyzes the de-methylesterification of pectin in plant cell walls during cell elongation.[1] Pectins are mainly composed of α(1, 4)-D-galacturonosyl acid units that are synthesized in a methylesterified form in the Golgi apparatus to prevent any interaction with Ca^{2+} ions during their intracellular transport.[2] The highly methylesterified pectins are then secreted into the apoplasm[3] and subsequently de-methylesterified in muro by PMEs. This can either induce the formation of pectin gels through the Ca^{2+} crosslinking of neighboring non-methylesterified chains or create substrates for pectin-degrading enzymes such as polygalacturonases and pectate lyases for the initiation of cell wall loosening.[4] PMEs belong to a large multigene family. Sixt-six PME-related genes are predicted in the Arabidopsis genome.[1] Among them, we have recently shown that AtPME3 (At3g14310), a major basic PME isoform in *A. thaliana*, is ubiquitously expressed in vascular tissues and play a role in adventitious rooting.[5] In flax (*Linum usitatissimum*), three genes encoding PMEs have been sequenced so far, including *LuPME3*, the ortholog of *AtPME3*. Analysis of the LuPME3 isoform brings new insights into the processing of these proteins.

LuPME3 is Expressed in Root Flax

In flax (*Linum usitatissimum*), three genes encoding PMEs have been sequenced so far: *LuPME1* (**AF355056**), *LuPME3* (**AF188895**) and *LuPME5* (**AF355057**).[6] The effects of the expression of the *LuPME3* gene, the ortholog of *AtPME3*, have been previously investigated in transformed flax cell lines (calli) and transformed tobacco. Both the methylesterification of pectins and the cellular cohesion were demonstrated to be affected in flax calli underexpressing *LuPME3*.[7] In transformed tobacco, *LuPME3* promoter was active mainly in immature leaves, roots and during pollen germination and pollen tube growth.[8] To investigate the expression pattern of *LuPME3* during the flax development, specific antibodies have been generated. In flax calli, as illustrated in **Figure 1A**, the antibodies recognized a single band. To confirm the specificity of the antibodies, an immunoblotting experiment was performed on cell wall-enriched protein extracts from flax calli transformed with a partial *LuPME3* sequence in an antisense orientation. The transformed calli showed very low level of expression of the corresponding transcripts.[7] At the protein level, as shown in **Figure 1A**, the immunoreactive band was no longer detected in the transformed calli, thus confirming that the antibodies specifically recognized LuPME3 in flax cell wall protein extracts. In addition, proteomic analysis of the immunodetected band, confirmed that the protein corresponded to LuPME3 (not shown). These antibodies were also shown to specifically recognize the Arabidopsis AtPME3 ortholog.[5]

To gain insights into the role of LuPME3 in flax, cell wall-enriched protein extracts from plantlets were separated by isolectric focusing (IEF) and then submitted to a PME activity assay on gel (zymogram) or to protein gel blot analysis. Flax seedlings were grown at 25°C for 3 d in the dark, then under light for 1, 7 and 13 d. Epicotyls (7 and 13 d only), cotyledons, hypocotyls and roots were collected and their cell wall proteins extracted. PME activity was detected on gel by the previously reported agar-pectin sandwich method.[9] As previously described,[10,11] flax seedlings expressed 2 neutral (N1 and N2), 4 basic (B1a, B1b, B3a and B3b) and 1 strongly basic PME forms (B2) (**Fig. 1B**). Protein Western analysis using anti-LuPME3 antibodies allowed the immunodetection of the B3a isoenzyme as the LuPME3 protein (not shown) among the various active PME spots. LuPME3 isozyme was found to be mainly active in roots, appearing progressively from 1 to 13 d (**Fig. 1B**). For confirmation, cell wall-enriched protein extracts from flax tissues were resolved on SDS-PAGE and immunodetected with the specific anti-LuPME3 serum after blotting (**Fig. 2C**). This corroborated the strong expression of the

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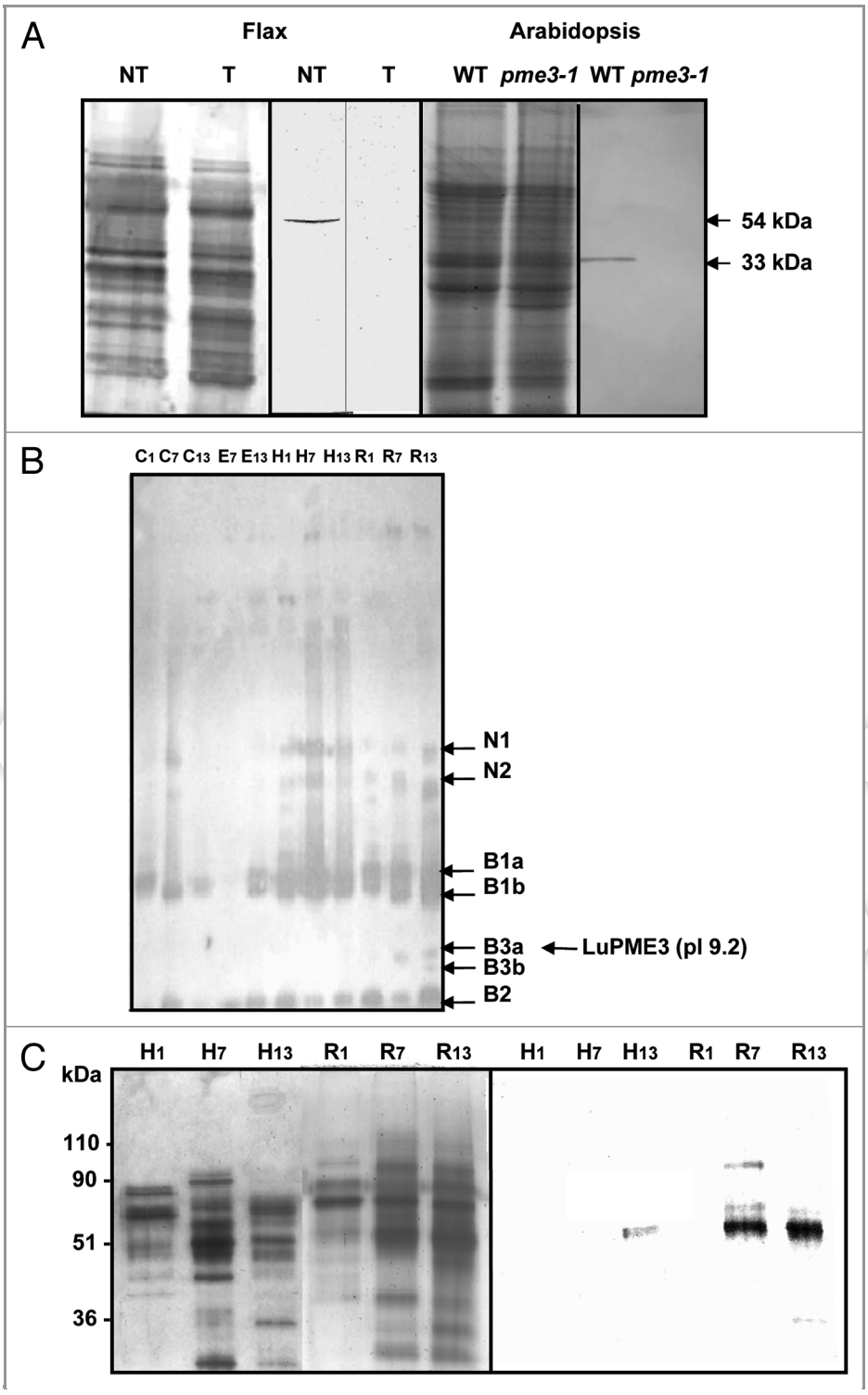

Figure 1. (A) SDS-PAGE and protein gel blot analysis using anti-LuPME3 antibodies of proteins extracted from the cell walls of flax calli and Arabidopsis plants. NT: Non transformed flax calli and T: Transformed flax calli underexpressing *LuPME3*. WT: wild-type Arabidopsis plants. *pme3–1*: Arabidopsis null mutant. Left panels: Coomassie staining. Right panels: protein gel blot. (B) Detection of PME activities (zymogram) of proteins isolated from cell walls of flax plant organs collected at various developmental stages and separated by IEF. (C) Silver staining (left panel) and protein gel blot (right panel) performed on flax cell wall enriched proteins separated by SDS-PAGE. C1, C7, C13: 1-, 7- and 13-d-old cotyledons. E7, E13: 7- and 13-d-old epicotyls. H1, H7, H13: 1-, 7- and 13-d-old hypocotyls and R1, R7, R13: 1-, 7- and 13-d-old roots.

LuPME3 protein in roots, as previously suspected from the analysis of the promoter activity observed in root vascular tissues and in root meristem of transgenic tobacco.[8] In conclusion, *LuPME3* encodes for an active basic PME, previously referred to B3a isoform, and is likely to play a major role in the flax root development. In that respect, LuPME3 and AtPME3 show strong similarities at the level of the protein sequence, the site of expression and physiological relevance.

LuPME3 Accumulates in Flax Roots as a Non Processed Protein

AtPME3 belongs to group 2 PMEs that are composed of an active domain and a N-terminal PRO domain separated by a proteolytic cleavage site.[1,12] This PRO region exhibits similarity with PME inhibitors and was proposed to prevent group 2 PMEs activity during their transport through the secretory pathway.[13] It has been speculated that the PRO region is cleaved from the PME domain during secretion as only proteins lacking this domain have been identified in plant cell walls.[14,15] This was recently confirmed by Wolf and collaborators[12] through the demonstration that the PRO region mediates the retention of unprocessed group 2 PMEs in the Golgi apparatus and that its cleavage is a prerequisite for secretion.

As its Arabidopsis ortholog, LuPME3 is synthesized as a group 2 pre pro-protein exhibiting the conserved RRLL motif required for its proteolytic processing.[6] From the prediction of the PRO domain and the cleavage site, LuPME3 pro-protein and mature protein are expected to exhibit MW of 54 kDa and 34 kDa and pI of 9.18 and 9.8, respectively. As illustrated in **Figure 1A and C**, anti-LuPME3 antibodies recognized a single polypeptide band exhibiting a MW of 50–54 kDa and a pI 9.2 in flax seedlings and callus which is compatible with the accumulation of a non-processed pro-protein in flax tissues. In contrast, AtPME3 accumulates in plant tissues as a mature PME (**Fig. 1A**), which was confirmed by

the identification of peptides mapping the PME3 domain in Arabidopsis cell wall-enriched fractions.[5] Absence of PME maturation is not a general feature in flax as LuPME5, another group 2 flax PME, was demonstrated to be fully processed in flax cells.[6] Such a difference in group 2 PME maturation is questionable. High molecular weight PMEs have also been purified from other plants although, in absence of sequence information about these esterases, it is highly speculative to conclude that the accumulation in tissues of such large PMEs also resulted from the deficiency in protein maturation.[16,17] In conclusion, the data presented in this addendum suggest that the maturation of group 2 PMEs by cleavage of its inhibiting PRO region is not a strict prerequisite for secretion and may differ depending on plant tissues and/or physiological conditions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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